Foraging behavior of parasitoid chalcid to the essential oil from bark of *Populus pseudo-simonii* ×*P. nigra* and *Quadraspidiotus gigas*

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Abstract: Four-armed airflow olfactometer was used to determining the foraging behavior of *Pteroptrix longgiclava* (Girault) (Hymenoptera: Aphelinidae) and *Encarsia gigas* (Tshumakova) (Hymenoptera: Aphelinidae) to the essential oils which emitted from the healthy bark of *Populus pseudo-simonii* × *P. nigra*, the infested bark injured by *Quadraspidiotus gigas* (Thiem & Gerneck), the body and scale of fixed 1st-instar-nymph of *Q. gigas*. The results from these experiments showed that the volatile oils produced from the injured bark and from the scale of fixed 1st-instar-nymph had a higher attractive ability to female adults of the two species of wasps. The essential oil produced from the scale of the pest at dosages of 3-7μL and the essential oil emitted by injured bark at dosages of 5-9 μL had a stronger alluring effect on the host searching behavior of *Pteroptrix longgiclava*. The essential oil from the body of fixed 1st-instar-nymph of *Q. gigas* also had certain effect on the host locating effort of *Pteroptrix longgiclava* and *Encarsia gigas*. Those two wasps did not shown any reaction to the essential oil produced by the healthy bark of poplar.

Keywords: *Populus pseudo-simonii* × *P. nigra; Quadraspidiotus gigas* (Thiem & Gerneck); *Pteroptrix longgiclava* (Girault); *Encarsia gigas* (Tshumakova); Essential Oil; Parasitoid; Foraging behavior

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Introduction

Successful parasitism by parasitoids of herbivorous insect host is preceded by several phases of host searching that lead females into the close vicinity of their potential host. In each of these phases the female often utilizes chemical stimuli to guide her in search for a suitable host. Chemical stimuli emitted by members of different trophic levels in the parasitoid's environment, such as the herbivorous host or the host's food plant, are often used in the host searching process *(Lewis et al.* 1984; Han *et al.*1999; Bai *et al.* 2001; Jacqueline *et al.* 1994).

Host derived stimuli are the most reliable indicators of the presence of host, because they can inform the parasitoids the presence, identity, availability and suitability of the host (Lewis *et al.* 1984; Liu *et al.* 2001; Noldus *et al.* 1988; Price *et al.* 1980; Tao *et al.* 2000; Vet *et al.* 1992; Vet *et al.* 1991; Xiao *et al.* 2000; Xu *et al* .2001, Xu Weian *et al.* 2000; Xu Zhaifu *et al.* 2000; Yan *et al.* 1999. Zhan *et al.* 2001; Zhang *et al.* 1998; Zhou *et al.* 2001). Although this type of stimuli has a high reliability, it is often hard to be detected at long distance (Vet *et al.* 1992, 1991). Two inherent constraints limit its use as stimuli for host location. Firstly, in terms of mass, hosts are small components of a complex environment and the information produced by them would be small in amount. Secondly, in long-term natural selection, the hosts have a fitting mechanism to avoid being parasitized. However, several types of host cues that are used by some parasitoids to locate hosts from long distances (Lewis *et al.* 1984; Liu *et al.* 2001; Noldus *et al.* 1988; Price *et al.* 1980; Vet *et al.* 1992; 1991).

Plant-derived stimuli are more detectable, because of their relatively large biomass, but they are less reliable to predict the presence of the host and the suitability of those hosts. The amount of herbivorous pest on the plant strongly affects the usefulness of the volatile cues from the plant. If the infestation is very high, information from the plant is very reliable, and can even replace the cues produced by the host pest (Price *et al.* 1980; Tao *et al.* 2000; Vet *et al.* 1991,1992; Xu Weian *et al.* 2000; Zhan *et al.* 2001).

Quadraspidiotus gigas (Thiem & Gerneck) is one of the most dangerous pests damaging the truck of poplar trees, and usually difficult to be controlled, because it is comparatively small and hidden under a waxiness scale. Five species of parasitic chalcid and 1 predator have been found in the poplar stands growing in the north region of China. The natural control abilities of *Pteroptrix longgiclava* (Girault) and *Encarsia gigas* (Tshumakova) are comparatively higher among those parasitoids. It seems that certain cues emitted by *Q. gigas* or by poplar trees were used by wasps in foraging to their hosts.

In order to understand the effect of essential oils released by poplar plant or by *Q. gigas* on the host finding of those parasitic chalcid, four-armed airflow olfactometer

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were used to determining the foraging behavior of wasps toward those volatile oils.

Material and methods

Materials

Pteroptrix longgiclava and Encarsia gigas

Barks of *Populus pseudo-simonii* × *P. nigra* infested by *Q. gigas* for more than 4 years was peeled, at the pupae stage of those parasitic chalcids, and was taken back to laboratory. Two to three kilograms of bark was put into a pasteboard bucket (40 cm in diameter, 40 cm high). This bucket was covered with a cardboard. A hole (5-6 cm in diameter) was made in the middle of this cardboard and filler was fixed onto this hole. A test tube was covered on the filler so that emerged chalcids can climb into the test tube. Those chalcids rearing bucket were kept in an animal rearing room at constant temperature of 25-28°C, under relative humidity of 60%-70% (L·D=16:8). The female adults of *Pteroptrix longgiclava* and *Encarsia gigas* were collected daily. Female adults were fed on the 20% sucrose water solution and kept in the animal rearing room. Twenty-four hour old female adult was used in the bioassays.

Essential oils

On July 10, 2000, at the fixed 1st-instar-nymph stage of *Q. gigas*, health poplar bark, and the infested bark by *Q. gigas* were collected from Lindian County, Heilongjiang Province. The 1st-instar-nymph of *Q. gigas* was brushed off from the bark using brush pen. Its body and waxiness scale were collected individually.

Healthy bark, infested bark, and body and scale of 1st-instar-nymph were put into adsorption system (Guo *et al.* 1997) individually to collect their volatile odor. The adsorbent tubes containing volatile odor substance were distilled using distilling equipment similar to the one used by Guo *et al.* (1997) and Yan *et al.* (1999), and dichloromethane was used as distilling solvent. Those essential oil distilled liquid was condensed to 2-mL under N_2 air-stream. Four kinds of essential oils were obtained. They are: 1) Essential oil from healthy bark (EOHB); 2) Essential oil from infested bark (EOIB); 3) Essential oil from the body of 1st-instar-nymph of *Q. gigas* (EOBFIN); 4) Essential oil from the scale of 1st-instar-nymph of *Q. gigas* (EOSFIN).

Methods

Bioassays

The four-armed airflow olfactometer used in this experiment is similar to the one used by Vet *et al.* (1991), with some modification by Ding *et al.* (1996). There are a circular central chamber and 4 testing jars on this olfactometer. The circular central chamber has a space of 2 000 cm^3 and cubage of each testing jar is about 250 cm^3. Those testing jars were connected to the circular central chamber with arms. Of the four jars, two were used for testing the attractive ability of those essential oils, and the other two jars were used as control ones. We took 4 pieces of (2cm×2cm) filter paper, piped certain dosages of testing volatiles onto two of them and dichloromethane onto the other two pieces of filter paper at the same dosage, and put those treated filter paper in the open air for 1 min to make the solvent volatilize. After then, all the filter paper was put into the testing jars. The testing wasps were put into the central chamber before the beginning of each experiment. The airflow speed is 1.5 L/min, and the airflow through the four arms was equalized by airflow-meter connected to each arm. The positions of odor sources were rotated after each replicate to avoid directional bias of those wasps. Three to six replications were tested for each odor in each dosage. All olfactometer apparatus were washed with detergent and rinsed with distilled water after each test. Each test was recorded for 15 min. Fifteen minute later, the number of wasps in each trapping jars and those still left in the central area were counted. Those wasps within odor jars were counted as responsive and those in control jars are counted as unresponsive ones. All the tests were conducted during 8:00 a.m. to 16:00 p.m., when wasps are most active in searching for the fixed 1-instar-nymph to lay their eggs naturally.

Data analysis

Responsive variable in volatile odor test were computed as follow:

Reactive Rate: $R = \frac{\sum Ni}{N} * 100\%$

Attractive Rate: $Ar = \sum Nti / N * 100\%$

Relative responsivity:

$$Rr = (\sum Nti - \sum Nci) / N * 100\%$$

Where, R is the reactive rate of those wasps acted in the testing, Ni is the number of wasps trapped in No. 'i' testing jar either with volatile oil in it or the control one, Ar is defined as attractive rate, Nti is number of wasps in No.'i' trapping jar with volatile odor in it, N is the total number of wasps used in each test, Rr is defined as relative responsiveness, and Nci is the number of wasps trapped in No. 'i' control jar.

Results

Attractive effect of different essential oils

To test the effect of the essential oils produced by healthy bark, infested bark, the body and scale of fixed 1st-instar-nymph of *Q. gigas* on the host searching of *Pteroptrix longgiclava* and *Encarsia gigas*, 5-μL dosage for each kind of essential oil were separately piped on the filter paper in the treating jars. The number of wasps reacted to those essential oils, the attractiveness of those oils and the relative responsivity of those wasps were listed in Table 1.

Table 1. The activeness of wasps to the essential oil from their herbivorous hosts or poplar barks

Wasp species	Repetition	5-μL EOHB			5-μL EOIB			5-μL EOBFIN			5-μL EOSFIN		
		R(%)	*Ar*(%)	*Rr*(%)	*R*(%)	*Ar*(%)	*Rr*(%)	*R*(%)	*Ar*(%)	*Rr*(%)	*R*(%)	*Ar*(%)	*Rr*(%)
	1	31.25	12.50	-6.25	75.00	56.25	37.50	68.18	31.82	-4.55	85.00	70.00	55.00
	2	23.53	11.76	0.00	73.68	52.63	31.58	55.00	25.00	-5.00	83.33	66.67	50.00
Pteroptrix	3	22.22	5.56	-11.11	72.73	54.55	36.36	60.00	40.00	20.00	93.75	87.50	81.25
longgiclava	4	23.81	19.05	14.29	66.67	57.14	47.62	70.59	47.06	23.53	88.24	64.71	41.18
(Girault)	5	41.18	17.65	-5.88	75.00	54.17	33.33	72.22	33.33	-5.56	73.68	63.16	52.63
	6	21.74	8.70	-4.35	78.95	57.89	36.84	76.47	52.94	29.41	88.24	76.47	64.71
	Average	27.29	12.54	-2.22	73.67	55.44	37.21	67.08	38.36	9.64	85.37	71.42	57.46
	1	42.11	21.05	0.00	71.43	50.00	28.57	70.83	37.50	4.17	94.44	77.78	61.11
	2	27.78	16.67	5.56	80.00	60.00	40.00	61.90	42.86	23.81	88.24	70.59	52.94
Encarsia	3	17.65	5.88	-5.88	75.00	50.00	25.00	70.00	40.00	10.00	71.43	66.67	61.90
gigas	4	27.27	18.18	9.09	58.82	47.06	35.29	66.67	38.10	9.52	82.61	65.22	47.83
(Tshumakova)	5	33.33	16.67	0.00	72.22	50.00	27.78	73.68	42.11	10.53	77.78	66.67	55.56
	6	25.00	8.33	-8.33	75.00	62.50	50.00	72.73	31.82	-9.09	78.95	68.42	57.89
	Average	28.86	14.46	0.07	72.08	53.26	34.44	69.30	38.73	8.16	82.24	69.22	56.21

Note: EOHB--Essential oil from healthy bark; EOIB--Essential oil from infested bark; EOBFIN--Essential oil from the body of 1st-instar-nymph of *Quadraspidiotus gigas*; EOSFIN--Essential oil from the scale of 1st-instar-nymph of *Quadraspidiotus gigas*. *R* is the reactive rate of those wasps acted in the testing; *Ar* is attractive rate; *Rr* is relative responsivity.

From the data in Table 1, it could be found that EOHB had little attractive effect on *Pteroptrix longgiclava* and *Encarsia gigas*. The average reactive rates (*R*) of these two wasps to EOHB were only 27.29% and 28.86%. This means that most of the wasps did not react to the EOHB odor resources. Most of the *Pteroptrix longgiclava* and *Encarsia gigas* reacted to the EOIB, EOBFIN and EOSFIN. The average reactive rate of *Pteroptrix longgiclava* to EOIB, EOBFIN and EOSFIN was 73.67%, 67.08% and 85.37% respectively, and similar result was obtained for *Encarsia gigas*. Of the four kinds of essential oils, EOSFIN was the most attractive one. Its average attractive rate (*Ar*) to *Pteroptrix longgiclava* and *Encarsia gigas* was up to 71.42% and 69.22% respectively. EOBFIN was inefficient in the attraction of the two species of wasps. Even though, more than 67% female wasp adults reacted to that volatile oil, but the attractive rate of EOBFIN to these two wasps was only 38.36% and 38.73. The relative responsivity (*Rr*) of EOBFIN to these two wasps was less than 10%, which indicated that a great number of stimulated female adults entered the controlling jars.

Influence of dosage on the attractive effect of essential oils

The dosage used in the bioassays usually has effect on the attractive ability of the volatile substance. To determining the behavior of *Pteroptrix longgiclava* to different dosage of EOHB, EOIB, EOBFIN and EOSFIN, 1-μL, 3-μL, 5-μL, 7-μL, 9-μL, 15-μL, 20-μL, 30-μL, 50-μL, and 100-μL each essential oil were piped on the filter paper which was going to be put in the trapping jars of olfactometer. Dichloromethane was used as control group. One minute after piping essential oils (and dichloromethane), the filter paper was put into those testing jars. Each treatment repeated 3 times. The number of parasitoids in each testing jars or in the circular central chamber was recorded after 15 min. The average reactive rate, the average attractive rate and the average relative responsivity were counted and drawn as Fig 1-4.

For the essential oil from the healthy bark (EOHB), the reactive rate of the *Pteroptrix longgiclava* female adult to the dosage of 1-100 μL was in range 22.0%-48.1%, the highest attractive rate of EOHIB was only 22.2% (at dosage of 15 μL) and the relative responsivity of this dosage was –3.7% (Fig. 1). That means more wasp went into the controlling jar rather than the jar with essential oil substance in.

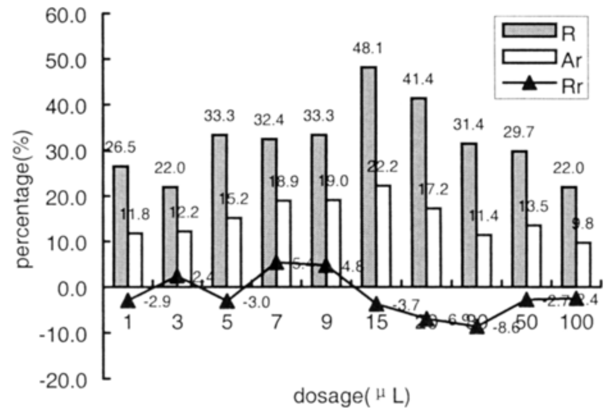

Fig.1 The reactive rate(*R*), attractive rate (*Ar*) and relative responsivity (*Rr*) of essential oil from the healthy bark to female adult of *Pteroptrix longgiclava* (Girault)

From Fig. 2, it was found that 5-μL, 7-μL and 9-μL EOIB

had higher attractive ability. The reactive rate of *Pteroptrix longgiclava* to those three dosages is 74.4%, 75.0% and 75.7% respectively, and the attractive rate is 53.8%, 54.5% and 59.5% respectively. Using less than 3-μL EOIB could decrease the attractive ability of this essential oil. The reactive rate was 59.5% and its attractive rate was 48.6, which were significantly less than that of 5-9 μL dosages. More than 15-μL EOIB could also restrain the attractive ability of this volatile oil. When 50-μL EOIB used in bioassay, only 47.4% female reacted to the volatile sources.

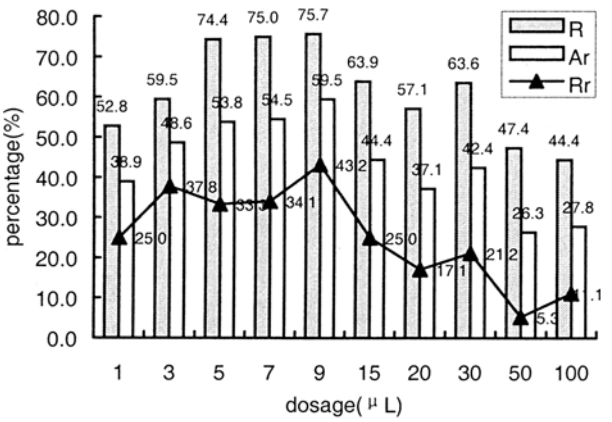

Fig. 2 The reactive rate (*R*), attractive rate (*Ar*) and relative responsivity (*Rr*)of essential oil from the infested bark to female adult of *Pteroptrix longgiclava* (Girault)

Fig. 3 showed that EOBFIN were not very attractive to female adults of *Pteroptrix longgiclava* for all the tested dosage (1-100 μL). When 5-μL dosage was used, 68.2% female adult reacted, but the attractive rate is only 40.9% and the relative responsivity was only 13.6%, which means that more reacted female adults of *Pteroptrix longgiclava* went into the control jars. The other dosage used in this experiments showed much less attractive ability than that of 5-μL dosage.

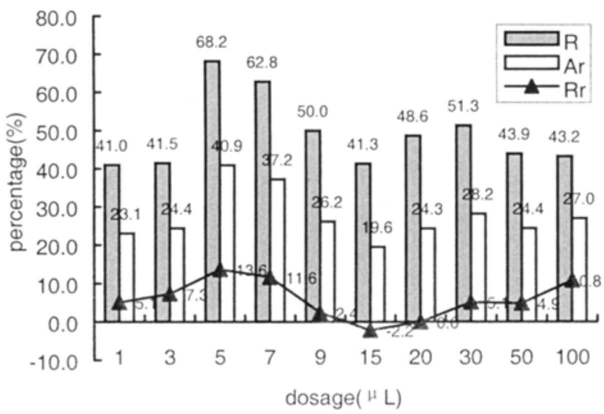

Fig. 3 The reactive rate(*R*), attractive rate(*Ar*) and relative responsivity (*Rr*)of essential oil from the body of fixed first instar nymph of *Quadraspidiotus gigas* (Thiem & Gerneck) to female adult of *Pteroptrix longgiclava* (Girault)

Fig. 4 showed that 3-μL, 5-μL and 7-μL of EOSFIN were more attractive to the female adults of *Pteroptrix longgiclava*. When 5-μL EOSFIN was used, 84.1% female adults reacted and 70.5% of them were attracted into the jars with essential oils in. When more than 15-μL EOSFIN was used, the reactive rate, the attractive rate and the relative responsivity declined quickly. The *R, Ar* and *Rr* at 15-μL dosage were 55.3%, 23.4 and –8.5% respectively and the *R, Ar* and *Rr* of 50-μL dosage were 38.1%, 19.0% and 0% respectively.

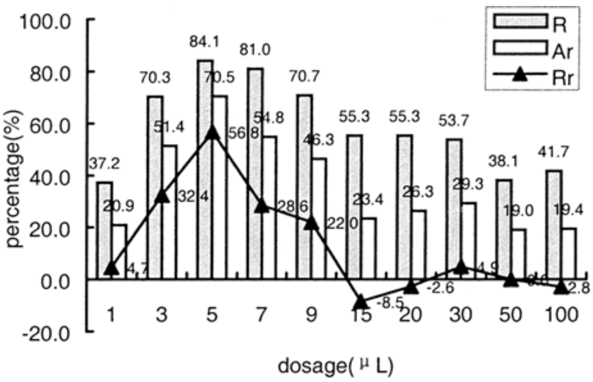

Fig. 4 The reactive rate (*R*), attractive rate (*Ar*) and relative responsivity (*Rr*) of essential oil from the scale of fixed first instar nymph of *Quadraspidiotus gigas* (Thiem & Gerneck) to female adult of *Pteroptrix longgiclava* (Girault)

Discussion

The essential oil from healthy bark (EOHB) at dosage of 5 μL had almost no attractive effect on the female adults of *Pteroptrix longgiclava* and *Encarsia gigas* in comparison with other kinds of essential oils used in experiment at the same dosage (Table 1). Experiment on different dosages (1-100μL) also showed that EOHB did not have attractive ability to *Pteroptrix longgiclava.* In contrast, EOIB, EOBFIN and EOSFIN were attractive to the two species of wasp. It suggests that the healthy bark of poplar did not emit any attractive chemicals to allure those adult wasps, but the infested bark, the body and scale of fixed 1st-instar-nymph of *Quadraspidiotus gigas* could emit some substances to guide those wasps in their searching of hosts.

EOIH at dosages of 5-9-μL and ECOSFIN at dosages of 3 -7-μL were more attractive to female adults of *Pteroptrix longgiclava* than at other dosages. When more than 15-uL EOIH or EOSFIN was used in the experiment, the reactive rate (*R*), attractive rate (*Ar*) and the relative responsivity (*Rr*) showed a sharp decreasetrend. This seems indicate that some of the substances emitted from the infested bark or from the scales of the fixed 1st-instar-nymph, when it is at high concentration, served as inhibitor for those wasp to recognize their host. On the other hand, since the cubage of this olfactometer used in the experiment was comparatively small, and the airflow was relatively slow, higher

dosage essential oils may make all of the olfactometer be filled with high concentration odor substances, thus resulting in failure of those wasps to find their way to searching host. To obtain the true scientific explanation for this phenomenon further experiment need to be carried out by wind-tunnel method.

Through our experiment it has been noticed that, in some cases, even though the reactive rate was higher, the attractive rate and the relative responsivity was comparatively low. For example, in the 9-uL EOSFIN treated group (Fig.4), the reactive rate is 70.7%, but its attractive rate and relative responsivity was only 46.3% and 22.0% respectively. It means that most of stimulated female adults went to the controlling jars. This phenomenon is possibly caused by the unsuitable higher concentration of the volatile odor substances or caused by some special compounds. Still the specific explanation for this phenomenon needs further research.

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